Validated Stability Indicating HPTLC of Diclofenac

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ABSTRACT

The present paper describes stability indicating high-performance thin-layer chromatography (HPTLC) assay method for Diclofenac in bulk drugs. The method employed TLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of toluene: methanol: triethylamine (6.5: 4.0: 0.1 v/v/v). The system was found to give compact spot for Aceclofenac (Rfvalue of 0.64 ± 0.028). Densitometric analysis of Diclofenac was carried out in the absorbance mode at 243 nm. The linear regression analysis data for the calibration plots showed good linear relationship with $r^2 = 0.999$ with respect to peak area in the concentration range 30 - 120 ng/spot. The developed HPTLC method was validated with respect to accuracy, precision, recovery and robustness. Also to determine related substance and assay determination of Diclofenac that can be used to evaluate the quality of regular production samples. The developed method can also be conveniently used for the assay determination of Diclofenac. The limits of detection and quantitation were 4.062 and 12.322 ng/spot, respectively by height.

Keywords: Diclofenac, validation, HPTLC

I. INTRODUCTION

Estimation of Diclofenac in Tablet by Proposed Method

- **Standard solution:** Working standard solution was prepared (10.0 μg/ml) as described under preparation of standard solution.

- **Sample solution:** Twenty tablets were weighed and average weight was calculated. Tablets were crushed to a fine powder. An accurately weighed quantity of tablet powder equivalent to about 10.0 mg of Diclofenac was shaken with about 8.0 ml of methanol, sonicated for 15 minutes, the volume was made up to 10.0 ml with methanol, and solution was filtered through Whatman Grade I filter paper. One ml of the filtrate was diluted to 100.0 ml with methanol to get concentration of 10.0 μg/ml (on labelled claim basis). Replicate sample solutions were prepared in similar manner.

- **Procedure:** Two bands of standard solution and six bands of sample solution of equal volume (5 μl) were applied on TLC plate and the plate was

developed and scanned as per optimized chromatographic conditions.

- **Calculation:** The instrument directly gives the weight of constituent in volume of sample solution applied by comparison with concentration of standard. This value was subsequently converted to percent of labelled claim using following formula.

Pulmoza tablet (Avg. wt.: 358.82 mg., Labelled claim: 200 mg per tablet)					
Sr. No.	Wt. of tablet powder taken (mg)	Amt. of Diclofenac estimated in applied 5 µL vol. (ng)		% of labelled claim	
		By Height	By Area	By Height*	By Area*
1.	14.50	41.07	40.96	100.66	100.26
2.	16.00	44.25	44.15	99.57	99.21
3.	18.30	50.83	50.94	99.91	100.11
4.	21.20	58.85	59.14	99.90	100.38
5.	22.50	62.99	62.79	99.86	99.44
* Each value is mean of five observations		Mean	99.90	99.90	
		±S.D.	0.364	0.497	
		% RSD	0.363	0.495	

Table: Results of estimation of Diclofenac in tablet

VALIDATION

❖ **Validation of the proposed method**

Validation of proposed method was ascertained on the basis of accuracy, precision, linearity & range, limit of detection, limit of quantitation, specificity, ruggedness and robustness.

❖ **Accuracy:** Accuracy of the proposed method was ascertained on the basis of recovery studies performed by standard addition method.

- **Standard solution:** Working standard solution was prepared (10.0 µg/ml) as described under preparation of standard solution.

- **Sample solution:** Accurately weighed quantities of pre-analyzed tablet powder equivalent to about 7.0 mg of Diclofenac were transferred to five different 10.0 ml volumetric flasks and 1.5 mg, 3.0 mg, 4.5 mg and 6.0 mg of standard Diclofenac added to 2nd, 3rd, 4th & 5th flask respectively (representing 70- 130% of labelled claim). This was followed by addition of methanol to make volume to about 8.0 ml in each flask, and the contents were shaken and sonicated for 15 minutes. Sufficient methanol was added to each flask to adjust the volume to 10.0 ml mark and filtered. One ml of each of the filtrate was diluted to 100.0 ml with methanol.

Calculation: Amount of Diclofenac (ng/5µl) obtained from instrument was converted to total Drug

Estimated by using following formula: $\quad T = \dfrac{Ew \times 1000}{Vs}$

The percent recovery was then calculated using the formula:

$$\% \text{ Recovery} = \dfrac{T - B}{C} \times 100$$

Where,

T	=	total drug estimated (mg)
E_W	=	Wt. (µg) of drug calculated by instrument in V_s
V_s	=	Volume (µl) of sample solution applied
B	=	amount of drug contributed by pre-analysed tablet powder (mg)
C	=	weight of pure drug added (mg)

Flask No.	Wt. of tablet powder taken (mg) + Amt of pure drug added (mg) (% of labelled claim)	Amt. of Diclofenac estimated in applied 5µL vol. (ng)		% Recovery	
	Pulmoza tablet (Avg. Wt.: 359.82 mg., Labelled claim: 200 mg per tablet)				
		By Height	By Area	By Height*	By Area*
1.	12.80 + 0 (70 %)	35.7	34.8	100.49	100.86
2.	12.60 + 1.5 (85 %)	41.4	42.6	99.87	100.08
3.	12.90 + 3.0 (100 %)	50.7	50.7	100.11	99.68
4.	12.70 + 4.5 (115 %)	56.9	56.1	98.96	98.87
5.	12.50 + 6.0 (130 %)	65.2	65.3	100.54	100.93
* Each value is mean of five observations			Mean	100.00	100.07
			±S.D.	0.632	0.872
			%RSD	0.632	0.872

Table: Results of recovery studies of Diclofenac in tablet

❖ Precision

• Repeatability

Precision of proposed method was ascertained by replicate analysis of homogeneous samples of tablet powder.

• Intermediate precision

The samples were analysed by proposed method on different days (intra-day & inter-day), and by different analysts.

Sr. No.	Observations	% of labelled claim					
		Intra-day		Inter-day		Different Analysts	
		By Height	By Area	By Height	By Area	By Height	By Area
1.	I	99.77	99.56	100.02	99.46	100.23	100.33
2.	II	99.95	99.35	99.78	99.25	99.52	99.92
3.	III	100.05	99.85	98.93	99.12	100.76	100.25
Mean*		99.92	99.70	99.58	99.28	100.18	100.16
±S.D.		0.141	0.256	0.572	0.173	0.622	0.216
% R.S.D.		0.141	0.252	0.578	0.173	0.622	0.216

Table: Result of precision studies

* Each value is mean of three observations

❖ Linearity and Range

- **Linearity of response**

Chromatographic response (peak height / peak area) as a function of concentration was studied.

- **Range of the method**

Sample weights of pre- analysed tablet powder were fortified by addition of standard drugs to have the range 70-130 % of labelled claim and the samples were processed as discussed under accuracy studies. The graph plotted as percent labelled claim vs. peak height or peak area.
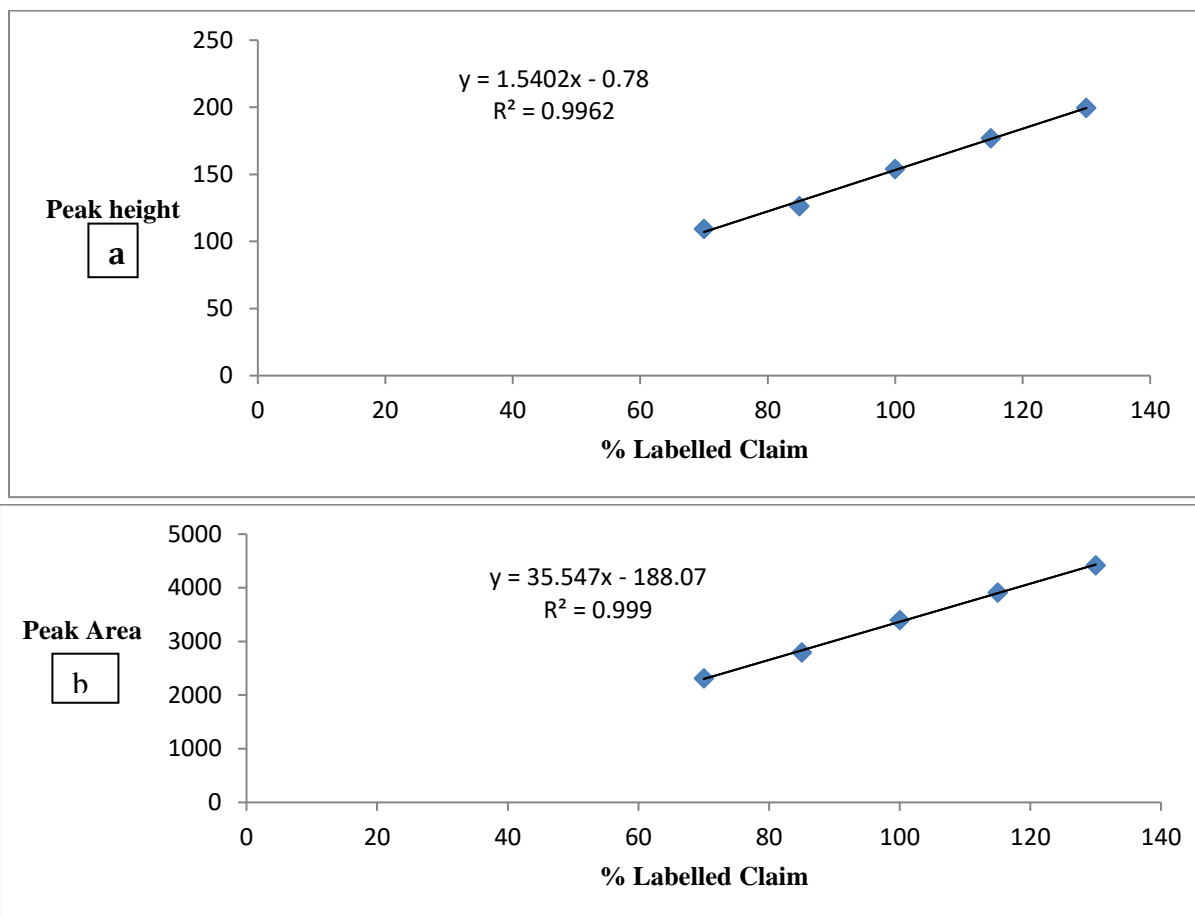

Figure 5: Calibration curve of range of method (a) by height (b) by area

Concentration range	70- 130% of labelled claim	
Parameter	Height	Area
Regression equation	Y=1.540X-0.78	Y=35.54-188.0
Slope	1.540	35.34
Y-intercept	(-) 0.78	(-) 188.0
Correlation coefficient	0.996	0.999

Table: Results of range of method

❖ **Limit of Detection (LOD) and Limit of Quantitation (LOQ)**

LOD and LOQ were determined by the method based on standard deviation of the response and the slope of calibration curve as per ICH guidelines and are as follows:

$$LOD = \frac{3.3\sigma}{S} \text{ And } LOQ = \frac{10\sigma}{S}$$

Signal to noise ratio (k) = 3.3 and 10 for LOD and LOQ respectively

σ = Standard deviation of response (Estimated by measuring the response in term of peak height or peak area of standard solution of conc. 30.0 ng/spot for five times and σ was calculated) = 1.455201, 48.71276 by height and area resp.

S = Slope of calibration curve (obtained from calibration curve) = 1.18, 60.86 by height and area respectively

S. No	Parameters	By Height	By Area
1.	LOD (ng/spot)	4.068	2.642
2.	LOQ (ng/spot)	12.334	8.005

Table: Results of LOD and LOQ studies

❖ **Solution State Stability and stability on plate**

The chromatograms of the same standard were obtained periodically over a period of 24 h.

Time (h)	Solution state stability		Stability on plate	
	Peak height*	Peak area*	Peak height*	Peak area*
1	151.96	3498.52	151.85	3498.62
3	152.14	3498.96	151.90	3498.21
7	152.36	3491.25	151.93	3495.54
24	151.99	3496.39	152.25	3495.95
Mean	152.11	3496.82	151.98	3497.08
± SD	0.183	3.536	0.181	1.560
% RSD	0.120	0.101	0.119	0.044

*mean of three observations

Table: Results of Solution State Stability and stability on plate

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Cite this article as :